Spectral in vivo fluorescence, photosynthetic activity and composition of phytoplankton in the Tropical Atlantic Ocean.

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Spectral *in vivo* fluorescence, phytoplankton composition and photosynthetic activity in the tropical Atlantic ocean

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Two different trophic situations in the tropical Atlantic Ocean: oligotrophic (st.02, 21°N; 31°W) and mesotrophic (st.M2, 18°N; 21°W) have been studied during the EUMELI 3 cruise (JGOFS- France, R/V "Atalante" 1-24/10/1991) with the general aim of quantifing fluxes of materia in the oceanic water column. The improvement of actual estimates of global oceanic production of carbon needs a better understanding of essential parameters of the photosynthetic process such as the quantum yield of growth and the absorption and fluorescence efficiency of chlorophyll a, as well as a more precise definition of the role played by the different primary producers. The respectif role of phycobilin containing and picoplanktonic organisms, for instance, could be underestimated at present owing to the lack of knowledge about their distribution (Lazzara, 1984) and ecophysiology. *In vivo* fluorescence spectra of chlorophyll a may be interpreted both as indicators of the whole photosynthetic pigments composition (Yentsch and Yentsch, 1979) and as correspondant to the PS2 photosynthesis action spectra of natural phytoplankton populations (Neori et al., 1986).

Fluorescence measurements have been carried out (Perkin-Elmer LS5B), both on the intact water sample (Fn) and after addition of DCMU 10^{-5}M (Fd), integrating the signal for about four minutes. Then the emission and the quantum corrected excitation spectra of fluorescence for chlorophyll a and phycoerythrin (PE), have been measured directly on suspensions, after a soft concentration on Nuclepore filters (0.22 ,µm) and using each filtered sample for blank substraction. This procedure avoids disturbance from Raman diffusion and by means of the quantum counter correction makes the spectra comparable with analogous measurements (Hofstraat et al., 1992). For pigment determination, 4 liters of seawater were filtered onto 47 mm Whatman GF/F glass fiber filters. After extraction in 100% methanol, chlorophylls and carotenoids were separated and quantified using the HPLC procedure described in Williams and Claustre (1991). Phytoplankton taxonomic composition of samples fixed with neutral formaldehyde (0.8%) has been analysed by phase contrast inverted microscopy. A preliminary analysis has been performed on samples which fluorescence spectra are shown hereafter. About 180 *in vivo* fluorescence spectra have been measured, which have allowed, even for oligotrophic waters and without any extraction procedure, a rapid distinction among different pigment compositions of phytoplankton populations.
At station 02 the chlorophyll a excitation spectra (fig. 1a), have their maxima at 450 nm owing to chlorophylls a, c and chlorophyll a_2 (*) and at 480 nm due to carotens, chl b_2 (*) and xanthophylls, the presence of phycobilins is not evident. The peak at 480 nm becomes progressively the main one for the deep samples (90-120 m) which show also a broad peak at 598 nm (chl b_2). Actually the phytoplankton population of the deep chlorophyll maximum layer has a pigment composition different from the surface one, with higher concentrations of chl a_2 and b_2, revealing the presence and relative importance of *Prochlorophyta* near the bottom of the euphotic zone (about 40% in terms of chlorophyll). With increasing depth, at 100 m, chl a_2 reaches the same concentration as chlorophyll a and chl b2 passes from 50% (100 m) to more than twice the concentration of chl a_2 at 140 m. The increase of chl b_2 beneath the maximum of chl a_2 is a constant characteristic of these profiles, for st. M2 as well.

At station M2, the chlorophyll a excitation spectra (fig. 1b) show the main peaks at 442 nm (mainly due to chlorophylls a, c and chl a_2) and at 475 nm (due to carotens and xantophylls). Moreover these spectra reveal the presence and photochemical activity of phycoerythrin with a peak at 545 nm, more evident for the surface phytoplankton and in the sub-thermocline maximum layer (35-40 m). The maximum of the spectra shifts from 442 nm at 20 m, to 475 nm for all the deeper samples, as previously observed for st 02, and seems to indicate an increasing role of the accessory pigments with depth.
Moreover fluorescence spectra of phycoerithrin (fig. 2) have been measured: they are characterized by two excitation peaks at 490 and 545 nm (em 590nm) and two emission ones at 575 and 682 nm (ex 490nm), and by the highest values within the phytoplankton maximum. Microscopic observations indicate as responsible for this PE content both coccoid cyanobacteria and *Cryptophyceae*. The presence of the latter is confirmed by HPLC analysis showing a significantly high concentration of alloxanthin, a xanthophyll exclusively present in this algal group, just in the phytoplankton maximum of this station.

In the surface samples (3m depth, c3-c9) along the route between Canary Islands and st. M2 (fig. 1c), the chlorophyll a excitation spectra become much more variable exibiting peaks at 440-450 and at 475 nm (due to chlorophyll a, b, c carotens and xanthophylls), at 490 and 545 nm (phycoerythrin) and also at 590 and 630 nm (phycocyanin and allophycocyanin) revealing the presence and the photochemical activity of several kinds of phycobilins in the surface oceanic phytoplankton. This is confirmed by the observations of Cryptophyceae in samples were the phycoerythrin peak is more evident (c7, c5) and of coccoid cyanobacteria present in variable amounts, but in every sample. Furthermore the presence of Oscillatoria erythrea (up to 27000 cells/dm^3) has been observed just in the samples which fluorescence show more evidently the presence of phycocyanin (c8, c3).

The primary productivity of these two sites can be estimated by measurements of normal (Fn) and after DCMU (Fd) fluorescence, the ratio Fd/Fn has shown relatively low values (between 1 and 1.6) in the mesotrophic station, whereas in the oligotrophic one it is frequently higher than 2 both in surface waters (10-30 m) and at depth, near the fluorescence maximum (80-120 m), but showing a high variability with time. To explore this variability the deep fluorescence maximum at st. 02, has been sampled every hour for 34 hours, following the same water mass by a drifting buoy and monitoring the vertical structure by CTD profiles. This time series of measurements allowed a periodic variation to be observed (fig. 3), in the photosynthetic activity of this deep phytoplankton population, much more evident at the depth of 80 m, with the highest values in the early morning and the lowest at dawn. The nature of the organisms responsible for this rythm has to be clarified.

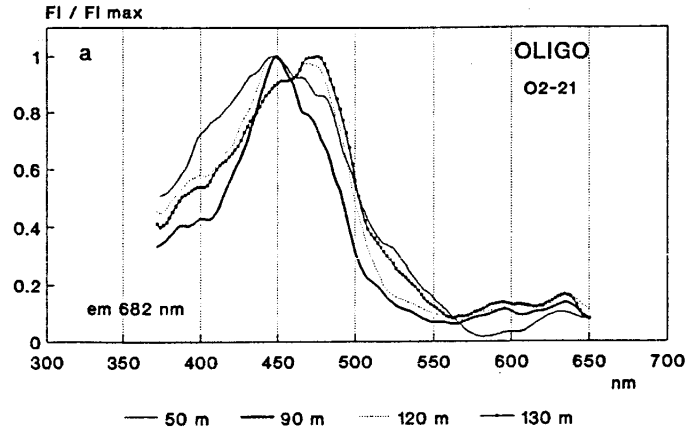
The two sites investigated show quite different environmental conditions: the mesotrophic one is characterized by a strong stratification and a sharp peak of phytoplankton biomass (chl a 2.8 mg m^{-3}) inside the thermocline (about 40 m) and by a relatively shallow euphotic zone (43-48 m for 1% of surface PAR). The oligotrophic site has a weaker and deeper thermocline, a vertical distribution of phytoplankton biomass with a deep maximum (100-120 m) reaching 0.5 mg m^{-3} of chl a and the 1% of surface PAR between 78 and 86 m.
Two different kinds of phytoplankton populations are characteristic of these diverse water columns and this is well reflected both by the different shapes of the fluorescence spectra and by the pigment and taxonomic compositions. The preliminary analysis of taxonomic composition of nano and micro-phytoplankton has shown: the dominance of coccoid cyanobacteria (mainly two forms with diameter less than 2 μm and 2-3, μm) reaching 70% of the total cells density in the biomass maximum of st. M2, the main difference being the higher number of species and cells present at st. M2 and the presence of *Cryptophyceae* only at the mesotrophic site. Such a presence is confirmed also by the

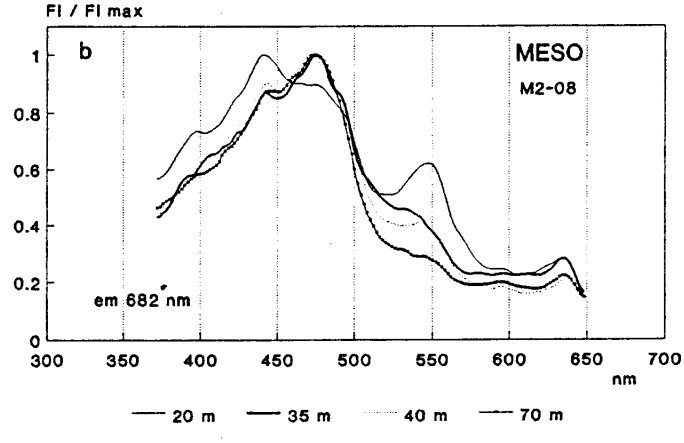
relative amount of alloxanthin to chlorophyll a (5-6%), the same as it has been found, in a spring bloom of *Cryptophyceae* along the coast of Britain (Klein and Sournia, 1987). Some species seem to be characteristic of both sites, diatoms like *Nitzschia sp. sec. Nitschiella* and N.*bicapitata*, dinoflagellates as *Oxytoxum variabile* and many *Gymnodiniaceae*, coccolitophorids like *Emiliania huxleyi*, *Discosphaera tubifer* and *Ophyaster hydroideus* and phytoflagellates (3-10 μm). The nano and micro-phytoplankton is more rich in species composition and more abundant in cells density at st. M2 (max 900000 cells/dm^3) compared with the oligotrophic site (max 50000 cells/dm^3) where the higher contribution to the total biomass is given by the *Prochlorophyta*, which were not taken in account by the microscopic observations but were evident both from HPLC and from cytofluorometric analysis (Morel et al., 1992).

The depth dependent change in fluorescence excitation spectra for both sites (shift of the main peak from 450 to 480 nm), p84 can be interpreted as an adaptation or acclimation to the low light conditions existing at the bottom of the euphotic zone. Both in tropical and in antartic waters some authors (Neori et al., 1984; Mitchell and Kiefer, 1984) have shown a similar spectral change in fluorescence and absorption of accessory pigments to be a photoadaptive response to low light regimes.

In conclusion, spectral *in vivo* fluorescence has shown a good correspondance with pigment and taxonomic analysis of phytoplankton and has proved to be able to rapidly characterize natural phytoplankton populations both for different trophic situations and depths. To understand the respectif roles of different size fractions and groups of organisms in the autotrophic carbon production of the oceans, seems to be of basic importance.

(*) the recently described divinyl-chlorophyll a and b (Gocricke and Repeta, 1992).





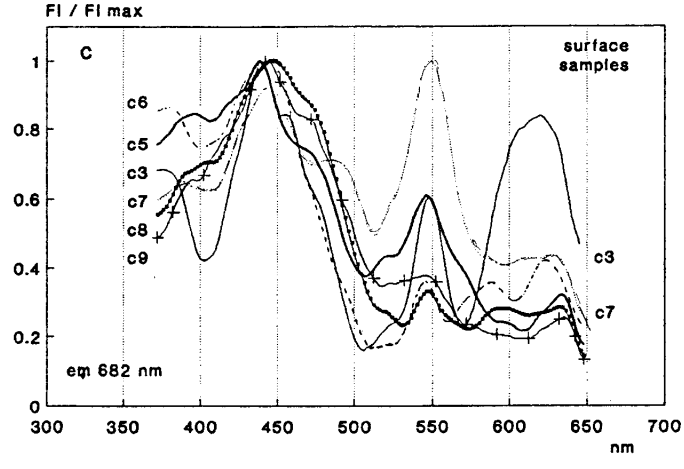


fig. 1 - Corrected excitation fluorescence spectra of chlorophyll a, *in vivo* (em 682 nm), at station 02-21 (a), M2-08 (b) and for surface (3m) samples c3-c9 collected during the route between st. M2 and Canary Islands (c). Fluorescence is normalized for the maximum.

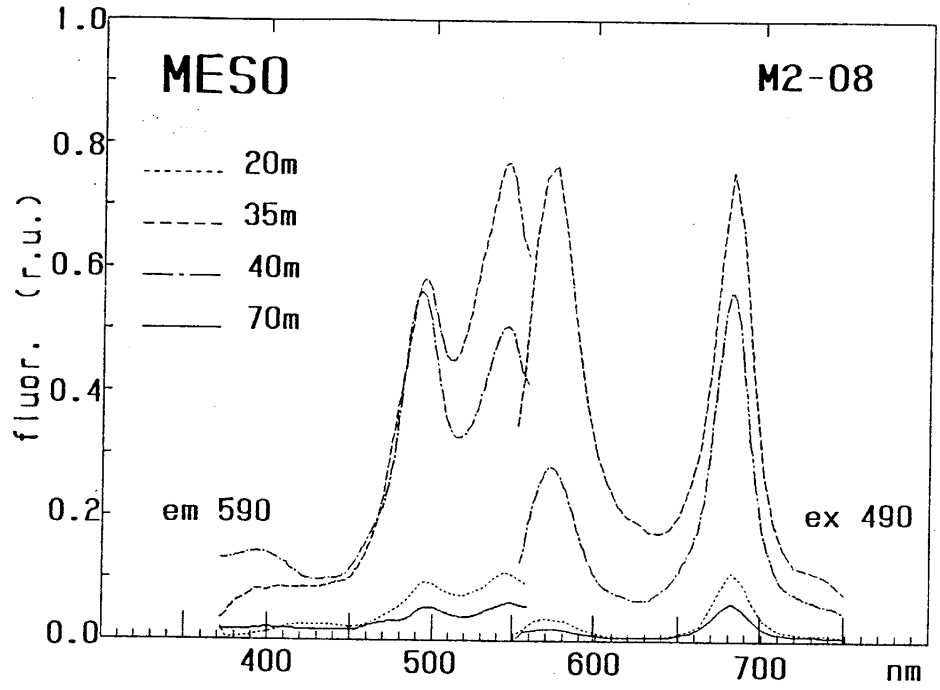


fig. 2 - Spectral fluorescence: excitation (em 590 nm) and emission (ex 490 nm) of phycoerythrin at station M2-08, in relative fluorescence units.

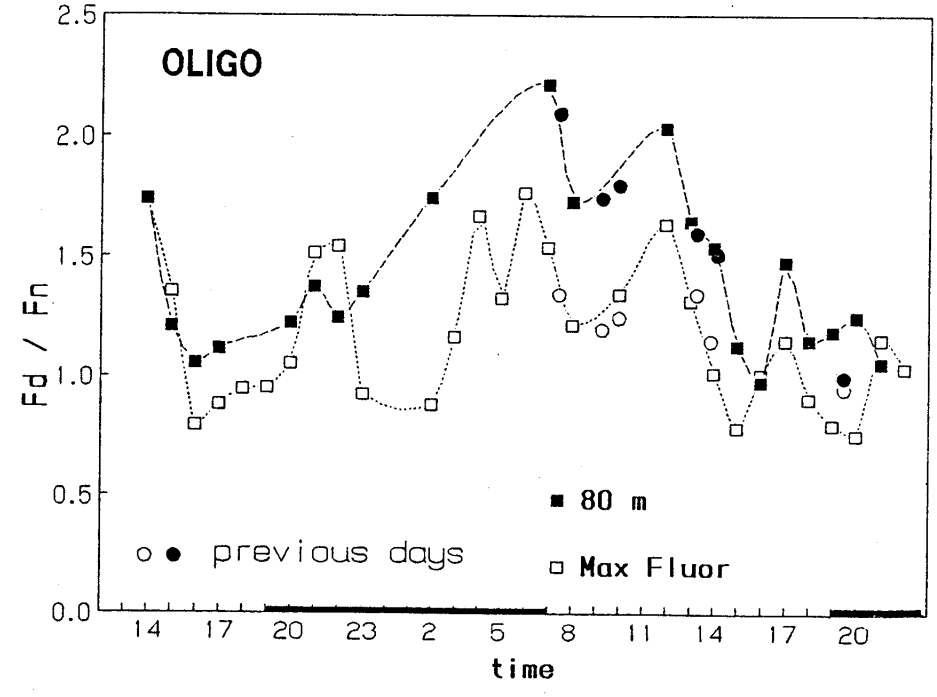


fig. 3 - Time series of variation for the Fd/Fn ratio (fluorescence with and without DCMU) at 80 m and at the fluorecence maximum of st. 02. Some measurements are added, made at st. 02 at the same depths, during the previous days.

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